

Michigan Department
of Community Health



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LabLink

Michigan Department of Community Health
Bureau of Laboratories

"Quality Laboratory Science for Healthier People and Communities"

Vol. 11 No. 1

Winter 2006

The Bugs Fight Back: VRSA, CA-VRSA and QRNG, Oh My!

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Again this January, a strain of vancomycin-resistant *Staphylococcus aureus* (VRSA) was recovered from a patient in Michigan, the fourth such isolate in Michigan of the six recognized worldwide. After the first two Michigan isolates, colleagues in other states commented on the skill and alertness of the Michigan clinical microbiology community. The concern now is how prevalent is this resistance.

It is not just VRSA that is troubling. There are numerous reports in the press of the prevalence of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA). College and professional sports teams in Michigan and elsewhere have developed awareness and respect for infection control practices, having learned this agent can shut down competitions and have devastating economic effects. Emergency rooms in many cities see cases of CA-MRSA every day. Many now routinely culture skin lesions that once were simply drained and treated with cephalosporins or quinolones.

A small project, conducted to confirm that quinolone-resistant *Neisseria gonorrhoeae* (QRNG) was not a problem in Michigan, detected 17 instances of resistance in 1122 (1.5%) isolates in the period from January

2003 thru September 2004. In 2005, 26 (3.4%) QRNG were detected from 764 surveillance isolates submitted from seven clinical labs and seven local health department Sexually Transmitted Diseases (STD) clinics. Three isolates submitted were determined to have intermediate resistance.

What is Michigan doing about this state of resistance? January 25, 2006 was the kick-off for a collaborative consumer education project between the CDC Foundation, the Michigan Antibiotic Resistance Reduction Coalition (MARR), and the Meijer® Pharmacy. Those returning unused/expired antibiotics to a Meijer's Pharmacy will receive a \$5.00 gift certificate and have their names entered in a drawing to receive a \$100 shopping spree at Meijer's.

January was also the start for a project aimed at developing new laboratory tools for QRNG surveillance. In collaboration with the San Francisco Health Department where the quinolone resistance approaches 25%, this study will examine the performance of a PCR-based test MDCH developed to detect resistance genes in samples collected for molecular probe testing. The goal is to replace the culture-based surveillance, which is labor-

intensive and expensive, with surveillance using specimens collected for routine non-culture testing.

MDCH BOL continues to support the role of clinical colleagues as the leading edge in detecting resistance and providing data needed to assess the state of resistance through sharing of antibiograms. BOL has funds that can be directed towards providing susceptibility standards documents and prepaid specimen submission shipping labels to clinical laboratories. (For more information, please contact Martha Boehme at 517-335-9654 or at boehmem@michigan.gov)

These circumstances illustrate that without the input of clinical partners, significant antibiotic resistance in Michigan would go unrecognized.

Infectious Disease Case Study in Michigan

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On June 13, 2005, a 60-year-old female from the Upper Peninsula, with a previous history of Lyme disease in 2002, presented to the ER with fever (99.9°F) of unknown origin, myalgia, nausea, headache, and right upper quadrant tenderness. The patient is a nonsmoker, drinks occasionally, and does not use illicit drugs. Examination revealed normal heart rate and rhythm and the lungs were clear. On June 20, the patient visited her primary care physician and complained of headaches, nausea, fever (100°F), myalgia, and stomach pains (pain level 7 out of 10) without vomiting or diarrhea. There was a faint, erythematous popular eruption on her abdomen. A Complete Blood Count (CBC), C-Reactive Protein (CRP), chemistry profile and other tests were ordered.

The patient was started on 100 mg 1 PO BID (orally twice daily) Doxycycline and pain medication. On June 22, the patient still experienced stomach pain and headaches but fever and muscle aches began subsiding. See Table 1 for laboratory test results.

TABLE 1
Laboratory Test Results

Test	Collected 6-13-05	Collected 6-20-02	Normal Range
WBC Count	2.6 L	6.4	4.5 – 10.8 K/mm ³
PMN'S	34.3 % L	80.5 % H	42-75%
Lymphs	47.8 %	14.1 %	12-50%
Monos	16.7 % H	5 %	1-12%
Platelet count	119	229	130-450 K/mm ³
Sodium	141	132 L	136-145 mmol/L
AST (SGOT)	81 H	66 H	8-42 U/L
ALT (SGPT)	89 H	79 H	0-65 U/L
CRP	Not done	18 H	0-0.3 mg/dL

1. What additional patient information would be helpful to obtain?
2. What is the differential diagnosis?
3. What other lab tests would assist in the diagnosis of this patient?

Part two will be presented in the next issue of the *LabLink* (Vol.11 No.2 Spring 2006).

Salmonella Serotyping 2001-2005

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Non-typhoid *Salmonella* spp. usually cause gastrointestinal infections accompanied by diarrhea, fever and abdominal cramps. It can cause localized infections or even septicemia in some individuals, particularly in the very young, the very old and the immunocompromised patients. There are some 2,500 recognized serotypes of *Salmonella*¹.

The top four serotypes in Michigan are consistent while the remainder of the top ten

strains depend on outbreaks occurring during any particular year (Table 1). Typically, the top two serotypes (Enteritidis and Typhimurium) account for approximately 40% of the strains received in this laboratory. The top ten serotypes normally account for 70-75% of the strains received. Serotyping *Salmonella* strains helps to identify outbreaks at an early stage. Further DNA fingerprinting using pulse field gel electrophoresis allows us to identify related strains of the same serotype.

Table 1. Predominant *Salmonella* Serotypes Isolated at MDCH 2001-2005

2001	2002	2003	2004	2005
Enteritidis 220 (24.7%)	Enteritidis 234 (24.6%)	Enteritidis 146 (17.5%)	Enteritidis 185 (21.8%)	Enteritidis 214 (21.2%)
Typhimurium 196 (22.0%)	Typhimurium 186 (19.5%)	Typhimurium 122 (14.6%)	Typhimurium 152 (17.9%)	Typhimurium 188 (18.7%)
Heidelberg 84 (9.4%)	Newport 79 (8.3%)	Newport 80 (9.6%)	Heidelberg 64 (7.5%)	Heidelberg 69 (6.8%)
Newport 50 (5.6%)	Heidelberg 57 (6.0%)	Heidelberg 58 (7.0%)	Newport 61 (7.2%)	Newport 68 (6.7%)
Java 35 (3.9%)	Anatum 53 (5.6%)	Oranienburg 27 (3.2%)	JAVIANA 22 (2.6%)	Oranienburg 36 (3.6%)
Oranienburg 19 (2.1%)	Java 27 (2.8%)	Saintpaul 27 (3.2%)	Paratyphil B 19 (2.2%)	Saintpaul 23 (2.3%)
Saintpaul 19 (2.1%)	Thompson 22 (2.3%)	Thompson 25 (3.0%)	Saintpaul 17 (2.0%)	Montevideo 16 (1.6%)
Thompson 19 (2.1%)	Infantis 18 (1.9%)	Muenchen 17 (2.0%)	Berta 16 (1.9%)	Schwarzengrund 16 (1.6%)
Berta 17 (2.1%)	Oranienburg 17 (1.8%)	Agona 16 (1.9%)	Oranienburg 16 (1.9%)	Stanley 14 (1.4%)
Muenchen 15 (1.7%)	Saintpaul 17 (1.8%)	Hadar 16 (1.9%)	Infantis 15 (1.8%)	Agona 11 (1.1%)

2001 - 70 serotypes were identified from 982 isolates submitted
 2002 - 85 serotypes were identified from 953 isolates submitted
 2003 - 78 serotypes were identified from 833 isolates submitted
 2004 - 81 serotypes were identified from 850 isolates submitted
 2005 - 87 serotypes were identified from 1008 isolates submitted

1. Bopp, C. A., Brenner F. W., Fields, P. I., Wells, J. G., and Stockbine, N. A., "Escherichia, Shigella, and Salmonella," *Manual of Clinical Microbiology*, Eighth Edition, 2003, ASM Press, Washington, D.C., pp. 654-671.

FUN FUNGI.....

Chlorella/Prototheca species

Sandy Arduin MT(ASCP) & Bruce Palma MT(ASCP) - Mycobacteriology/Mycology Unit

A 59-year old white female with history of hypertension, type II diabetes, and ulcerations of the anterior and posterior calf developed gangrene in the fourth and fifth toes of her right foot.

Specimens from this area were collected and submitted to the hospital laboratory for routine aerobic and fungal culture. The aerobic culture yielded *Staphylococcus aureus* and *Klebsiella pneumoniae*. Growth from the fungal culture was submitted to MDCH for identification.

The referred culture was received on a SAB agar slant and had green yeast-like growth. Microscopically, spherical sporangium filled with numerous, round endospores were found. The culture microscopically resembled *Prototheca* spp. The presence of green pigment ruled out the achlorophyllic species *Prototheca*. MDCH suspected that the organism was a *Chlorella* species. A subculture of the original slant was sent to Dr. Jerry Brand, director of the University of Texas Culture Collection of Algae for confirmation. Dr. Brand confirmed the identification of this culture as *Chlorella* species.

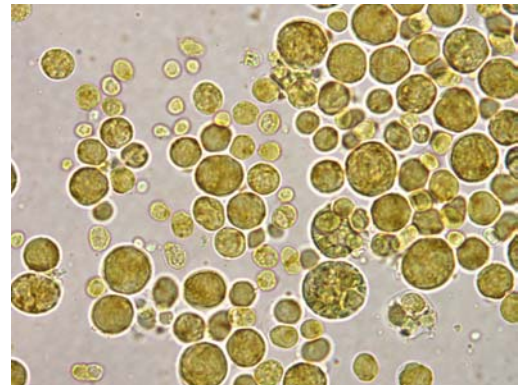
Dr. Brand provides the following information on the habitat of *Chlorella* species. "*Chlorella* spp. are often found in freshwaters and in soils, especially where organic compounds are plentiful. Some *Chlorella* species and related genera grow in specialized habitats. For example, various species related to *Chlorella vulgaris* grow in the bodies of invertebrates, and may even survive for long periods of time within intracellular vesicles of some invertebrates."

Very little information is available on infection by *Chlorella* spp. or other green algae. Almost all cases of infection by green algae have been in animals (e.g., cattle, sheep and beaver). Only one case of green algae infection in humans has been reported (2,5).

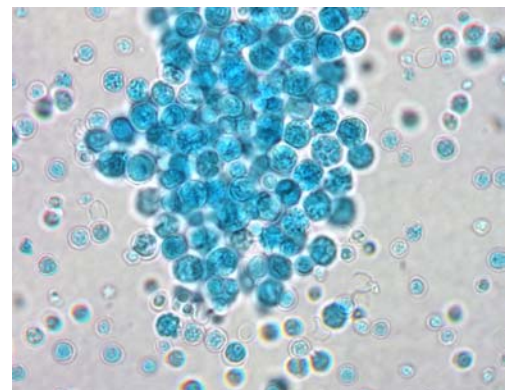
Chlorella spp. resemble *Prototheca* spp. microscopically, but can be distinguished from *Prototheca* by the presence of intracellular

chloroplasts. Macroscopically, *Prototheca* spp. are cream colored, while *Chlorella* spp. are bright green in color.

Prototheca spp. is found in aqueous locales including lakes, streams, ponds, and even tap water. It has been isolated from feces and soil. Infection typically occurs in an abrasion or cut to the skin that has been exposed to contaminated water. Patients typically present with an isolated nodule. Ulceration may or may not be present. Patients that are severely immunocompromised may develop disseminated disease. Infection is typically caused by *Prototheca wickerhamii*, but may occasionally be caused by *P. zopfii*. Protothecosis is a rare infection. Fewer than 100 cases have been reported in the United States since initially reported in 1964. Protothecosis is commonly found in severely immunocompromised people.



***Chlorella* spp.**



***Prototheca* spp.**

References:

1. de Hoog, G.S., Guarro, J., Figueras, Gene & M.J. 2000. *Atlas of Clinical Fungi*, 2nd Ed. Centraalbureau voor Schimmelcultures. Utrecht, The Netherlands.
2. Jones, J.W., H.W. McFadden, F.W.Chandler , W.Kaplan, and D.H.Connor. 1983. *Green Algal Infection In A Human*. American Journal of Clinical Pathology. 80:102-107.
3. Kurtzman, C.P., Fell, J.W. *The Yeasts, A Taxonomic Study*. Elsevier. New York.
4. Lee, Wie-shing, Lagios, Michael, Leonards, Richard. *Wound Infection by Prototheca wickerhamii, a Saprophytic Alga Pathogenic for Man*. Journal of Clinical Microbiology. July 1975. pp62-66.
5. Pfaller, M.A., Diekema, D.J. 2005. *Unusual Fungal and Pseudofungal Infections of Humans*. Journal of Clinical Microbiology. 43:1495-1504.
6. www.emedicine.com/derm/topic348.htm

This Issues Picture Quiz: What Mould Is This?



Colonies are yellowish-white, velvety to powdery in texture. Microscopically globose conidia are borne on pegs all over the surface of the hyphae. As the conidia mature the underlying hyphae undergoes lysis.



Michigan Rabies Working Group Hosts Conference

April 24, 2006 is the date of the Michigan Rabies Conference. This conference will feature:

- Morning plenary on emerging raccoon strain rabies in the Midwest.
- Nationally recognized speakers.
- Breakout sessions for public health and environmental components of rabies surveillance, prevention and control.

This conference is open to all public health professionals, healthcare providers, veterinarians, animal control, wildlife biologists and control personnel at no cost.

For registration and more information please visit:

www.mi.gov/documents/MRC_SaveDate_145377_7.pdf

MDCH BOL Customer Satisfaction Survey Results

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As part of the strategic plan, the MDCH Bureau of Laboratories (BOL) sought feedback from both clinical and public health customers on the laboratory support unit, testing services and overall customer service.

An on-line survey was developed to solicit feedback from customers between October 27 and December 6. The 14-question survey was promoted through fax, email, and the winter *LabLink*. Forty-two clinical and twelve public health customers completed the survey (total = 54). There was no way to determine how many customers were invited to participate as respondents were asked to forward the survey to others. Because respondents were self-selected, it was not a random survey.

When asked, "How could MDCH BOL improve our service?" 25 responded. Most suggestions for improvement were related to customer service and communication issues.

When orders for 100 collection kits were filled by the laboratory support unit, a separate written survey was included with the order. Thirty-four surveys were returned and no major problems were revealed. Fifteen percent of survey respondents praised staff member Ron Dietz for his outstanding customer service (we agree!). Congratulations, Ron!

Clerical staff also polled callers during a two-day period and found that nearly 80% of the callers were transferred. As a result of this survey, internal phone service training will be expanded within the BOL to reduce the number of transfers needed to reach the right department.

Results of the survey are presented in Tables 1 and 2 (Page 7). Several respondents (44%) indicated that information is not easily obtained from the Bureau of Laboratories web site. Further comments are invited as BOL looks at ways to improve.

The current guide to laboratory services is located at <http://www.michigan.gov/mdchlab>. Click on "Laboratory Services Guide." The guide contains lists and links to detailed descriptions of the tests available at MDCH BOL. Lists are arranged both alphabetically and by laboratory testing sections and units. All have printer-friendly versions.

Respondents were asked to comment on "What is the one thing that MDCH BOL does well?" Of thirty-two comments received, ten mentioned that laboratory staff are helpful and responsive to callers. The AFB/Mycology, Reference Bacteriology, and Virology/HIV Units all received positive comments. Training and Outreach efforts were also mentioned as helpful services.

The MDCH BOL wishes to thank all those who took the time to respond to the surveys. The responses and feedback have been presented to both the MDCH Continuous Quality Improvement Team and the Action Core Team and will be used to develop goals and priorities in the strategic planning process. This will assure that BOL customers notice improvements in the future.

If you have additional feedback on quality and services, please contact Sam Davis, Quality Assurance Officer, at 517-335-8074 or at DavisSam@michigan.gov.

Table 1. MDCH BOL Customer Satisfaction Survey Results

	Always	Most of the time	Sometimes	Almost never	Never	I do not use the website
It is easy to obtain information about MDCH laboratory services from the MDCH website www.Michigan.gov/mdchlab	8.77%	47.37%	10.53%	0.00%	0.00%	33.33%
It is easy to find out the status of a test in progress.	23.64%	49.09%	25.45%	1.82%	0.00%	
When testing is not performed (e.g., a specimen is rejected), the reason is clearly explained.	49.09%	43.64%	7.27%	0.00%	0.00%	
Test results are received within an acceptable timeframe.	18.18%	69.09%	12.73%	0.00%	0.00%	
Test reports are easy to interpret.	45.45%	50.91%	3.64%	0.00%	0.00%	
I am easily able to contact the people I need at MDCH.	30.91%	52.73%	14.55%	1.81%	0.00%	
The people at MDCH respond quickly to my requests for service or assistance.	43.64%	47.27%	7.27%	1.82%	0.00%	

Table 2. MDCH BOL Customer Satisfaction Survey Results (continued)

	Completely Agree	Generally Agree	Generally Disagree	Completely Disagree
I am confident in the quality of testing services provided by the MDCH Bureau of Laboratories.	64.41%	35.59%	0.00%	0.00%
The test menu offered by MDCH meets my expectations for a state public health laboratory.	54.24%	45.76%	0.00%	0.00%
MDCH test requisitions are simple to complete.	28.81%	62.71%	8.48%	0.00%

Bureau of Laboratories Vision

The Bureau of Laboratories is a stronger, more diverse team within and integrated public health system. We utilize advanced technology and innovative leadership to provide comprehensive public health services in our dynamic global community.

Bureau of Laboratories Mission

We are dedicated to continuing leadership in providing quality laboratory science for healthier people and communities through partnerships, communication and technical innovation.

Quirky Bugs...

Bartonella henselae

Stephen Haskell, BS, SM(ASCP)
Reference Bacteriology Unit

Frequently, requests are received in the reference bacteriology unit at MDCH, to confirm or rule out a highly infectious agent such as *Brucella* species. *Brucella*, being highly infectious and frequently implicated in laboratory-acquired infections, requires enhanced biosafety precautions for handling. Due to its clinical significance, the processing, testing and reporting is streamlined to reduce turn around time. One such request, led to the identification of a totally different and highly unusual bacterium.

An 81-year-old male, who had previously undergone a heart valve replacement, became ill one week after having dental work. He was hospitalized with a high fever and had a diagnosis of endocarditis. Blood cultures were ordered along with serological tests. Reports showed that serological tests for fungal, *Coxiella*, and *Ehrlichia* antibodies were negative, but *Brucella* IgG was positive at a high titer. Blood cultures became positive after five days incubation. All six cultures were growing tiny colonies of small to medium gram-negative rods. *Brucella* was suspected and the isolate was submitted to the MDCH laboratory for confirmation. *Brucella* species was ruled out within two hours upon specimen receipt.

Two distinct colony types grew, an adherent (or pitting), entire, convex, gray moist colony and an adherent, irregular, molar tooth, cream white colony. The organism was inactive for carbohydrate metabolism, oxidase negative and catalase positive. Cell wall fatty acids analyses by GLC showed an unusual pattern and were consistent with a rare isolate called *Bartonella henselae*. This identification was later confirmed at CDC by PCR testing. Members of *Bartonella* species are closely

related to *Brucella* on the basis of 16s ribosomal RNA comparison but are not easily confused with *Brucella* species as the characteristic phenotypic differences in these groups are striking.

Bartonella henselae is the causative agent of cat scratch disease (CSD), a self-limiting infectious disease in healthy individuals that usually resolves spontaneously over 2-5 months period. CSD is characterized by edema and pain in the lymph nodes. It causes bacteremia, endocarditis, bacillary angiomatosis and bacillary peliosis primarily in immunocompromised hosts. *Bartonella henselae* is globally endemic in cats with the prevalence of antibodies much higher in warm humid climates. Bacteremia rates in cats can vary but tend to be higher among feral animals in many areas. Bacteremia has been documented in healthy domestic cats that have been specifically associated with bacillary angiomatosis or cat scratch disease in their human contacts.

The first description of CSD is credited to Henri Parinaud, who referenced the condition in French medical literature in 1889. Dr Robert Debré was the first to recognize the cat as a vector for this disorder and coined the term cat scratch disease in 1931. About 2000 hospital admissions, with CSD, are reported yearly in the United States. Prevalence is approximately 6.6 cases per 100,000 persons. Symptoms can vary from mild to severe and may include malaise, anorexia, or both. Approximately 75% of cases occur from September through March. In 80-90% of cases, patients are younger than 21 years. The genus *Bartonella* was first described in 1913 and referred to the erythrocyte-adherent organisms originally

described by Dr. A.L. Barton in 1909. The unification of the genera *Bartonella* and *Rochalimaea*, as single genus did not take

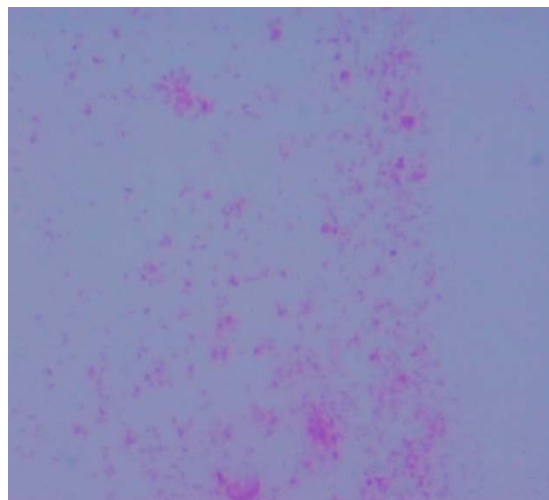
place until 1993. The 1995 merger of *Grahamella* with *Bartonella* completed the changes to date (Table 1).

Table 1

Species of the Genus <i>Bartonella</i>		
Validly Named species	Type species	Basonym
<i>Bartonella bacilliformis</i>	Type	
<i>Bartonella doshiae</i>	New species	
<i>Bartonella elizabethae</i>	New combination	<i>Rochalimaea elizabethae</i>
<i>Bartonella grahamii</i>	New species	
<i>Bartonella henselae</i>	New combination	<i>Rochalimaea henselae</i>
<i>Bartonella quitana</i>	New combination	<i>Rochalimaea quitana</i>
<i>Bartonella taylorii</i>		
<i>Bartonella vinsonii</i>	New combination	<i>Rochalimaea vinsonii</i>
Valid Names for Which 16-S rDNA Sequences Are Not Yet Available		Basonym
<i>Bartonella clarridgeiae</i>	New species	
<i>Bartonella peromysci</i>	New combination	<i>Grahamella peromysci</i>
<i>Bartonella talpae</i>	New combination	<i>Grahamella talpae</i>
<i>Bartonella vinsonii</i> Subsp. <i>berkhoffii</i>	New combination	<i>Rochalimaea vinsonii</i>
<i>Bartonella vinsonii</i> Subsp. <i>vinsonii</i>	New combination	<i>Rochalimaea vinsonii</i>



Bartonella henselae on 5 % sheeps blood agar after 5 days incubation at 35 C° in 10 % CO₂. Scant growth adherent to the agar surface and tiny colonies are of two types a smooth convex and a molar tooth like colony type.



Gram stain of *Bartonella*: Faintly stained gram negative pleomorphic rods.

LabLink is published quarterly by the Michigan Department of Community Health, Bureau of Laboratories, to provide laboratory information to Michigan health professionals and the public health community.

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DCH-0096